Convenient diagnosis of spinal and bulbar muscular atrophy using a microchip electrophoresis system

Hirofumi Maruyama1, Hiroyuki Morino2, Yuishin Izumi2, Kouichi Noda3, Hideshi Kawakami1

1Department of Epidemiology, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan; 2Department of Neurology, Tokushima University Hospital, Tokushima, Japan; 3Department of Neurology, Higashihiroshima Medical Center, Higashihiroshima, Japan

Received December 5, 2012; Accepted January 15, 2013; Epub March 8, 2013; Published March 18, 2013

Abstract: Spinal and bulbar muscular atrophy (SBMA) is a slowly progressive motor neuron disease. Lower and primary sensory neuronopathy is one of the major neuropathological changes that occurs in SBMA. However, many signs are common to SBMA and amyotrophic lateral sclerosis (ALS), and SBMA patients are sometimes diagnosed with ALS. Leuprorelin may be used to treat SBMA, but an accurate diagnosis is necessary for treatment and care. Genetic diagnosis can be performed to detect the expansion of a CAG repeat in the androgen receptor gene in SBMA patients. To screen for this expansion, we used a microchip electrophoresis system. The discrepancy between the actual repeat length and that found by the microchip electrophoresis system was roughly dependent on the repeat length. The mean difference was –6.8 base pairs (bp) in SBMA patients, –0.30 bp in controls. The microchip electrophoresis results were approximately 2 CAG repeats shorter than the actual repeat length in SBMA patients. Using this method, we screened our ALS samples (31 were familial, 271 were sporadic): 4 subjects were diagnosed with SBMA; 2 had familial ALS, and 2 had sporadic ALS (0.7%). The microchip electrophoresis system is semi-quantitative, convenient and useful for screening a large number of samples.

Keywords: Spinal and bulbar muscular atrophy, androgen receptor, CAG repeat, microchip electrophoresis, amyotrophic lateral sclerosis

Introduction

Spinal and bulbar muscular atrophy (SBMA) is characterized by mid-life onset, is slowly progressive [1], has sex-linked recessive inheritance and shows proximal spinal and bulbar muscular weakness [2]. Contraction fasciculation, hyporeflexia, finger tremor and gynecomastia are characteristic of SBMA. The upper motor neurons are not affected. Lower motor and primary sensory neuronopathy is one of the major neuropathological changes in SBMA [3]. Mild androgen insensitivity and reduced fertility have also been noted [4]. However, it is difficult to diagnose SBMA when the disease is at an early stage or when inheritance is not clear. In addition, many signs are common to SBMA and amyotrophic lateral sclerosis (ALS). This clinical heterogeneity makes an accurate diagnosis difficult when typical symptoms are not present [5]. ALS also has a mid-life onset and is characterized by upper and lower motor neurons involvement; without intensive physical support, approximately 50% of patients die within 3 years of onset [6]. However, patients with mild ALS progression have been identified [7].

Therapeutically, androgen therapy was initially attempted for SBMA [8, 9], but the effect was not maintained. In transgenic mouse model of SBMA, symptoms were markedly exacerbated by testosterone administration in female, and phenotypes were dramatically rescued by castration in male [10]. Leuprorelin, a luteinizing hormone-releasing hormone agonist that reduces testosterone release from the testis, rescued phenotypes in a transgenic mouse model of SBMA [11]. In a clinical trial, leuprorelin positively effected for swallowing in patients with disease durations of less than 10 years [12]. This result indicates that early diagnosis is necessary if leuprorelin is to be used to improve SBMA symptoms. SBMA should not be miss-
Diagnosis of spinal and bulbar muscular atrophy

Diagnosed as ALS; therefore, genetic diagnosis is required.

SBMA is caused by an increase in the CAG repeat in the first exon of the androgen receptor (AR) gene [13]. Nuclear inclusions containing the mutant and truncated AR with expanded polyglutamine have been detected in the motor neurons in the brainstem and spinal cord [14]. Genetic diagnosis could be performed to detect this CAG repeat expansion. The normal repeat number is highly polymorphic but is less than 34, whereas the pathological repeat number is 38 or more [http://www.ncbi.nlm.nih.gov/books/NBK1333/], and the number of repeats does not overlap. Initially, CAG repeat expansion was detected using radiolabelled polymerase chain reaction (PCR) products, which is complicated and time-consuming procedure. Subsequently, the color capillary electrophoresis was used, which is costly. Here we report the convenient diagnosis of SBMA using PCR and a microchip electrophoresis system. This method is semi-quantitative and useful for screening, especially for a large number of samples.

Materials and methods

Subjects

The research procedure was approved by the Ethics Committee of Hiroshima University. All examinations were performed after obtaining informed consent from the patients or their families. The patients were recruited from neurology clinics across Japan. The subjects included 32 SBMA patients and 54 control subjects.

Detection of the triplet repeat expansion

Expansions of the CAG repeats in AR were identified using PCR. Up primer (5'-ACCTACCGGCA-CCCAGAG-3') and Low primer (5'-CTCATCCAGG-ACCAGGTAGC-3') are used. The following PCR cycling parameters were used: an initial denaturation for 5 min at 95°C; 10 cycles consisting of 1 min at 95°C, 1 min at 60°C, and 1min at 72°C; 15 cycles consisting of 1 min at 95°C, 1 min at 55°C, and 1min at 72°C; 15 cycles consisting of 1 min at 95°C, 1 min at 50°C, and 1min at 72°C; and a final extension of 5 min at 72°C.

The precise repeat length was determined by sequencing the PCR product using Applied Biosystems BigDye terminator v3.1 sequencing chemistry with an ABI PRISM 3130 Genetic Analyzer (Foster City, CA, USA).

The PCR product was analyzed using the Shimadzu Microchip electrophoresis system MultiNA (Kyoto, Japan). All samples were analyzed in triplicate. The difference between the length determined using microchip electrophoresis and actual length estimated by sequencing was calculated.

Screening the CAG expansion in an ALS cohort

We screened CAG expansions to identify SBMA in clinically diagnosed male ALS subjects. Familial ALS (FALS), which was defined as ALS in subjects who had a positive family history, was 31 subjects, and sporadic ALS (SALS) was 271 subjects.

Results

Distribution of the actual CAG repeat length

The distribution of the actual CAG repeat length is illustrated in Figure 1. The range of the CAG repeat length was 43 to 62 in SBMA patients (mean; 48.0 repeats) and 14 to 33 in controls (mean; 23.2 repeats).

Discrepancy between microchip electrophoresis and sequencing

The distribution of discrepancies between the microchip detection size and the actual length is shown in Figure 2. The mean differences were –6.8 bp (SD: 3.31) in the SBMA group and –0.30 bp (SD: 2.66) in the control group. The microchip electrophoresis results were approximately 2 CAG repeats shorter than the actual repeat number in SBMA patients. There was no overlap in repeat number between SBMA patients and controls.

Screening of the CAG expansion in the ALS cohort

We identified 4 subjects who had a CAG expansion in the androgen receptor: 2 of these subjects were FALS subgroup (2/31, 6%) and 2 were sporadic ALS subgroup (2/271, 0.7%). Patients 1 and 2 had positive family histories, and their brothers had muscle disturbance. Patients 3 and 4 were sporadic. Patient 1 developed muscle weakness when he was 58 years old. He felt dyspnea, and exhibited restrict-
Diagnosis of spinal and bulbar muscular atrophy

Figure 1. Distribution of the actual CAG repeat length. The SBMA data are indicated by the solid bar, and control data are indicated by the vertical line bars.

Figure 2. Distribution of the discrepancies. SBMA data are indicated by the solid triangle, and control data are indicated by the white circle. The horizontal line shows the position at which there are zero discrepancies. The vertical axis indicates the size of the discrepancy (base pair; bp).

In humans, leuprorelin decreased mutant AR accumulation [18], decreased serum creatine kinase levels and may rescue the early phase of SBMA [11]. Therefore, it is important to diagnose SBMA in the early stage. In the early stage of ALS, clinical and electromyogram signs include progressive lower motor neuron dysfunction in the arms, legs, and face; by contrast, upper motor neuron signs are often equivocal [5]. Therefore, some SBMA patients may be difficult to distinguish from ALS patients; therefore, genetic diagnosis is important.
Diagnosis of spinal and bulbar muscular atrophy

Parboosingh reported that 2% of patients diagnosed with ALS actually suffer from SBMA [5]. We identified 4 subjects who had expanded CAG repeat lengths in AR in the ALS cohort. SBMA diagnosis is difficult if the patient does not have a family history or gynecomastia. Treatment may soon be available for SBMA, and patients who show muscle atrophy should be screened for CAG repeat lengths in AR. Specifically, male FALS subjects whose brothers also have ALS but whose parents do not have ALS should be screened.

The limitation of this system is accurate determination of the repeat size. The number of repeats influences the clinical expression of SBMA, with a trend toward earlier onset and more severe disease as the repeat length increases [19, 20]. We previously reported that using gel electrophoresis, the measured repeat length is more variable for higher CAG repeats number [21]. Likewise, the microchip electrophoresis results were 2 CAG repeats shorter than the actual repeat number in SBMA patients. Therefore, if the repeat number determined by microchip electrophoresis is approximately 35, the repeat number should be determined by sequencing.

In conclusion, the microchip electrophoresis system may provide a convenient diagnosis of SBMA. We also show that SBMA patients can be mis-diagnosed with ALS. It is important to distinguish SBMA from ALS because most ALS is rapidly fatal, and SBMA may be able to be treated in the near future. We should not be mis-diagnose SBMA as ALS; therefore, genetic diagnosis is required. The microchip electrophoresis procedure is semi-quantitative and useful for screening a large number of samples.

Acknowledgement

This work is supported by Funding Program for Next Generation Word-Leading Researchers from Cabinet Office, Government of Japan.

Conflict of interest statement

There is no conflict of interest.

Reference

Diagnosis of spinal and bulbar muscular atrophy


